suggested the subtle interaction of PRG4 with HA can be influenced by sample preparation, storage conditions, and presence of other molecules.
These results contribute to the understanding of rhPRG4's aggregation and interaction with HA, which can have functional consequences and therefore provides the framework for the development of potential new rhPRG4+HA containing biotherapeutics for the treatment of osteoarthritis and other conditions.

1112-Pos Board B63
Thermodynamic and Hydrodynamic Examination of ClpB Assembly
JiaBei Lin, Aaron L. Lucius.
Chemistry, University of Alabama at Birmingham, Birmingham, AL, USA.
E. coli ClpB is a heat shock protein that belongs to the AAA+ protein family. Studies have shown that ClpB and its eukaryotic homologue, Hsp104, can disaggregate denatured proteins by themselves or cooperate with the DnaK chaperone system in vivo. It is thought that ClpB requires binding of nucleoside triphosphate to assemble into hexameric rings with protein binding activity and ClpB majorly exist as hexamer in the presence of nucleoside triphosphate. In contrast to this conclusion, our sedimentation velocity data show that ClpB can form hexamer in the absence of nucleotide and ClpB resides in a monomer-dimer-tetramer-hexamer equilibrium in the presence of ATPgS (a slowly hydrolysable ATP analog). ClpB hexamers exhibit fast subunits exchange in the absence of nucleoside triphosphate, while the exchange rates decrease in the presence of a large excess of ATPgS. We anticipate our studies on ClpB assembly to be a starting point of for understanding how ClpB hexamers disaggregate protein aggregates. For example, knowing the population of ClpB hexamers in solution is essential for the interpretation of it is only possible to study the energetics and kinetics data for the of ClpB catalyzed disaggregation processreaction by knowing the population of ClpB hexamer in solution.

1113-Pos Board B64
Structural Study of AIPL1: Molecular Basis of its Function and its Role in Blinding Diseases
Chitrak Gupta[1], Abigail Hayes[2], Mark D. Distefano[3], Visvanathan Ramamurthy[4], Blake Mertz[1].
[1]Chemistry, West Virginia University, Morgantown, WV, USA, [2]Biochemistry, West Virginia University School of Medicine, Morgantown, WV, USA, [3]Chemistry, University of Minnesota, Minneapolis, MN, USA, [4]Ophthalmology, West Virginia University School of Medicine, Morgantown, WV, USA.
Leber's congenital amaurosis (LCA) is a severe form of childhood blindness, and mutations in the putative chaperone AIPL1 have been linked to LCA [1]. It is known that AIPL1 interacts with phosphodiesterase 6 (PDE6), a protein involved in visual phototransduction, in both rod and cone cells [2]. Recently, small-angle X-ray scattering experiments combined with homology modeling revealed the sub-domain arrangements of AIPL1 [3]. However, the molecular mechanism of the interaction between AIPL1 and PDE6 remains poorly understood due to a lack of high-resolution structural data. The interaction of AIPL1 and PDE6 is believed to be mediated by a prenyl group that is introduced at the C-terminus of PDE6 as a post-translational modification. To test this hypothesis we have employed a combined experimental and computational approach. Molecular dynamics (MD) simulations were carried out on the AIPL1 homology model, identifying the principal components that lead to large-scale conformational fluctuations. Subsequent MD studies will be carried out to identify the differences between wild-type AIPL1 and mutants of AIPL1 in their interaction with a C-terminal prenylated fragment of PDE6. Fluorescence studies will also be carried out to characterize the difference in binding of labeled prenyl ligands with several variants: wild-type AIPL1, mutants of AIPL1, and a mouse version of Aipl1, which lacks the C-terminal domain found in human AIPL1. Our studies will provide a biophysical understanding of the processes underlying this binding. These results have the promise to provide valuable insight into the role of AIPL1 in normal vision, and in blinding diseases that affect humans. [1] Sohocki, Nat. Gen., 24:79 (2000); [2] Ramamurthy, PNAS, 100:12630 (2003) Kolandaivelu, JBC, 284:30853 (2009) Kolandaivelu, HMG, 23(4):1002 (2014); [3] Majumder, J. Biol. Chem, 288:21320 (2013).

1114-Pos Board B65
Novel Approaches to the Study of Protein-Protein Interface Properties
Mihaly Mezei.
Structural and Chemical Biology, Icahn School of Medicine at Mount Sinai, New York, NY, USA.
Over 1000 protein-protein complexes extracted from the Protein Data Base (PDB) are investigated. The interface is defined by the parameter-free definition based on mutual proximity. Surface atoms are identified using a combination of the solvent-accessible surface and the circular variance (shown earlier to be capable of characterizing the degree of burial of atoms in a macromolecule). Surface properties will be defined using contact residues, geometric properties defined with the help of circular variance and solvation properties defined using simulations in the grand-canonical ensemble. It is expected that the information gathered in this work will help select the right model among the (usually) several plausible predictions from the currently available protein-protein interaction prediction calculations.

1115-Pos Board B66
Sedimentation in a Time-Varying Centrifugal Field for Rapid Attainment of Sedimentation Equilibrium
Peter Schuck, Michael Metrick, Huaying Zhao.
NIH, Bethesda, MD, USA.
Sedimentation equilibrium (SE) analytical ultracentrifugation is a gold standard for the rigorous thermodynamic study of buoyant molecular weight and reversible interactions of macromolecules in solution. A significant drawback is the long experiment time, as it takes days to attain SE with standard solution columns. We have developed a new method for using a time-varying centrifugal field optimized such as to attain SE in significantly shorter time than usually required. Experimental data show that this permits long-column SE experiments to be carried out in times comparable to sedimentation velocity experiments, approximately fivefold shorter than standard SE. In contrast to the classical initial overspeeding method, which uses a single initial speed, we employ a freely varying rotor speed profile during an initial phase, for example, parameterized as a step-wise modulated exponential decay to the desired SE rotor speed. The rotor speed schedule is computationally optimized on the basis of numerical Lamm equation solutions for given macromolecular sedimentation parameter estimates, with the goal to provide a rapid attainment of equilibrium without the drawback of strong transient sample pre-concentration at the base of the solution column. The resulting rotor speed schedule frequently includes both over- and under-speeding sequences, and can be conveniently implemented on the Optima XLA/I analytical ultracentrifuge. We extended AUC data analysis models in SEDFIT to permit the analysis of concentration profiles in arbitrarily time-varying fields, to make it possible to exploit the migration in the initially high centrifugal field for estimates on macromolecular sedimentation parameters, which may be used in real-time to refine the prediction of the rotor speed schedule, so that the SE experiment can be optimized in both information content and time efficiency.

1116-Pos Board B67
ATP Binding is Prerequisite to the Helical Structure of Human Rad51 Presynaptic Filament
Judit Fidy[1], Bálint Borka[1], Eva Bulyaki[2], Jozsef Kardos[2], Gusztáv Schay[1].
[1]Department of Biophysics and Radiation Biology, Semmelweis University Budapest, Budapest, Hungary, [2]Department of Biochemistry, Eötvös Lorand University, Budapest, Hungary.
Human Rad51 is a key protein component of homologous recombination, the error free repair process of double strand DNA breaks. It is a 37 kDa protein of two domains. The full structure of the protein is not known in atomic details, X-ray crystallography has been successful only in the case of the larger C-domain in complex with a regulatory protein, BRCA2. The structure of the N-domain has been determined by NMR. It is known that in the repair process, the recombinases form a helical filament around ssDNA overhangs - the presynaptic filament (PSF) - at the double strand breaks, the structure of which is of vital significance in the proceeding of the repair. It is also known that the recombinases possess ATPase activity, and ATP is needed for the successful repair. While in the case of the bacterial-, archeal,- and yeast - homologues of HsRad51, data were presented concerning the parameters of their ATP-dependent PSF structures in crystalline conditions, such results are non-existent for HsRad. In this study our goal was to provide evidence for the purely structural role of ATP in forming the PSF structure by HsRad51. Therefore, the hydrolysis of ATP has been excluded by adding Ca to the solutions instead of Mg. The topology of HsRad structures without and with added ATP/Ca and 75mer ssDNA has been determined by transmission electron microscopy. The formations were labeled by the fluorescent dye ANS, and pressure perturbation fluorescence spectroscopy has been applied to characterize the strength of interaction at protomeric interfaces in the Rad51 filaments. We found that the formation of the ordered filamentous structure clearly requires the presence of ATP/Ca, and that the interface binding strength is the highest in the presynaptic filament of helical structure.